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Gastric Microbiota and Resistance to Antibiotics

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Abstract

Studies on gastric microbiota find several bacterial families and species in the stomach using molecular-based techniques. When biopsies are cultured, there may be growth of bacteria, pure culture of *Helicobacter pylori*, or no growth. When looking at the histological sections of corresponding biopsies no bacteria may be seen, except curved rods (*H. pylori*) adherent to the gastric epithelial cells. In a number of biopsies, several different bacteria are cultured with or without *H. pylori*. The non-*H. pylori* bacteria cultured are like the normal oral flora and may be contamination of the samples during endoscopy. In histological sections, these bacteria are seen above the mucin layer and not adherent to the epithelial cells confirming that it is contamination of the samples and can thus not be regarded as gastric microbiota. Therefore, the susceptibility of *H. pylori* to antibiotics is independent of coexisting bacterial flora. A review of *H. pylori* susceptibility to antibiotics in untreated and previous treated patients will be given including meta-analyses of *H. pylori* susceptibility to metronidazole (MTZ), clarithromycin, and levofloxacin. These data indicate that these antibiotics become more doubtful to use for primary therapy and should be banned for secondary therapy without susceptibility testing.

Keywords: gastric microbiota, *H. pylori*, histology, susceptibility testing, resistant rates

1. Introduction

Microbiota and microbiome are not always clearly defined or distinguished. The human microbiota comprises the population of microbial species that live on or in the human body. This is the resident flora of the body and does not include the transient flora (sampling contamination, etc.).

The microbiome is constituted by all the genes inside these microbial cells and is thus restricted to detection by molecular methods (sequencing, polymerase chain reactions [PCR]) [1].

By molecular methods, bacteria are usually identified to family and genera level [2]. Bacterial families and genera may include species and types of bacteria that may have completely opposite actions in the human body [3]. It is, therefore, doubtful if molecular methods alone are sensitive enough to predict the effect of the composition of microbiota. The limited original literature on gastric microbiota has mainly focused on gastric cancer and contains conflicting results [4–7]. There are many difficulties in investigating the gastric microbiota. One thing many authors are not aware of is the difficulty of getting samples without contaminating bacterial flora (**Figure 1**) [8]. In animal models, the whole stomach can be removed, and contamination of the stomach can be avoided, but in most animal species, physiology, acidity, etc. of the stomach are very different from the human stomach. Samples from the human stomach are usually taken as biopsies during gastroscopy. Even though the endoscope and the forceps are sterilized or decontaminated, it will be contaminated with oral bacterial flora during gastroscopy and thereby will the samples be contaminated by oral flora mainly of the phyla *Firmicutes* [8, 9].

Bacterial resistance to antibiotics can occur either if the bacteria obtain plasmids containing resistance genes from other bacteria in the microbiota (conjugation); they can take

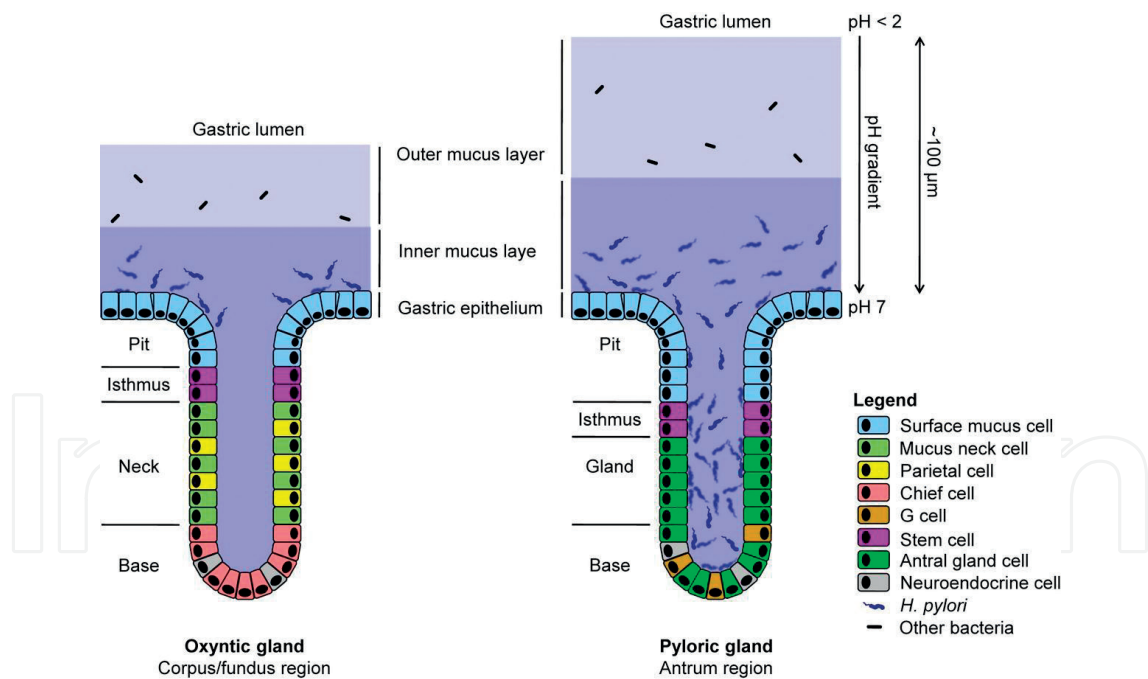


Figure 1. Schematic illustration of the gastric mucosa with the main cell types of oxyntic and pyloric glands in the gastric epithelium. Gastric stem cells reside in the isthmus zone of the gland and differentiate into precursors of the different cell lineages, which migrate either apically toward the gastric lumen or downwards to the base. The superficial epithelium and the gastric glands are covered by a viscous mucus layer mainly composed of MUC5AC, secreted by the SMCs, and MUC6, secreted mainly by MNCs and antral gland cells. The mucus layer consists of an inner layer, which is firmly attached to the epithelium, and an outer loose layer. The gastric pathogen *Helicobacter pylori* has been shown to use the transmucus pH gradient between the acidic gastric lumen and the near-neutral epithelial surface for spatial orientation to reach its niche at the juxtamucosal epithelium. The precise location of non-*H. pylori* microbiota is still hypothetical. [8].

up free DNA with resistance genes from the environment (transcription) or DNA can be transferred by bacteriophages (transduction). Furthermore, mutations can occur in the bacterial genome which may result in resistance if the mutation occurs in the part of the genome that codes for a structure on which the antibiotics act; this action may be interfered, and the bacteria becomes resistant to the antibiotic [10–12]. The conjugation of plasmids increases with the number of different bacteria in the microbiota and depends on a close contact between the bacteria. Uptake of free DNA does not demand a direct contact with other bacteria, but bacteria should probably be present in the close environment [3]. Mutations occur in all bacteria with a certain time because of natural replication errors [12]. Some bacteria mutate more often than others; but because of the short generation time for bacteria, each bacterial clone will have several mutations. If the mutation occurs in a part of the genome, which is target for the antibiotics, resistance to the antibiotic may occur.

2. Study on gastric microbiota

In a previous unpublished study that included 411 biopsies from patients undergoing upper gastrointestinal endoscopy were investigated both by microaerobic culture and by histology (Table 1). From 249 (60%) biopsies other bacteria than *H. pylori* were cultured. These bacteria were oral flora, that is, *Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Neisseria* spp., etc., which may indicate contamination of both the endoscope and the biopsies during the procedure. In histological sections, very few bacteria except *H. pylori* were seen in 20 (5%) of the biopsies. In all cases, the bacteria were located superficial to the mucus layer and not in relation to the epithelial cells and *H. pylori*, which confirm that it is contamination from the oral cavity. The discrepancy in the number of biopsies with other bacteria than *H. pylori* between culture and histology may be because very few bacteria (less than 5 colonies) are cultured and the preparation of histological sections may remove much of the mucin and the contaminating bacteria. *H. pylori* was found alone without contamination in 60 biopsies by culture and in 83 biopsies by histology which indicate that *H. pylori* is a true gastric microbiota (Figure 2).

All known mechanisms for *H. pylori* resistance to all antibiotics are point mutations located on the chromosome (Table 2), indicating no uptake of plasmids or free DNA, which support that *H. pylori* is the only bacteria in the true gastric microbiota and everything else is transient contaminating flora [13].

No. of biopsies	Culture		Histology	
	<i>H. pylori</i>	Other bacteria	<i>H. pylori</i>	Other bacteria
411	106	249	83	20

Table 1. Comparison of culture and histological finding of *H. pylori* and other bacteria (oral flora) in gastric biopsies.

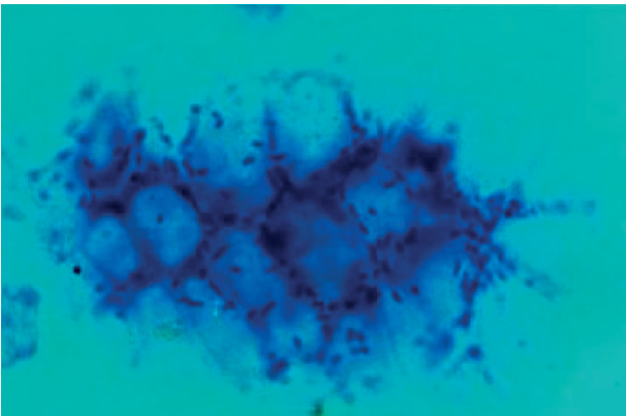


Figure 2. Imprint cytology showing the presence of *H. pylori* (Giemsa stain, ×400) Rahbar [84].

Resistance to	Mutation
Amoxicillin	PBP1
Clarithromycin	InfB
	rp1V
	A2142C
	A2142G
Metronidazole	A2143G
	rdxA
	frxA
	fdxB
Fluoroquinolones	gyrA
	gyrB
Tetracycline	AGA925-967TTC
Rifampicin	RNA polymerase subunit beta/beta

Table 2. Examples of mutations in *H. pylori* causing resistance to antibiotics.

3. Diagnosis of *H. pylori*

The detection of *H. pylori* can be done by invasive and noninvasive methods. The invasive methods require a biopsy, whereas the noninvasive methods are gentler for the patient.

Culture of *H. pylori* may be difficult and the sensitivity may be rather low (50–85%) [14]. The sensitivity of the culture depends on transport time to the lab and the culture method used [15]. Different agar plates or incubation time can also give different results on the same biopsy. Two biopsies from the antrum and two biopsies from the fundus are preferred when making a culture as *H. pylori* is unevenly distributed in the stomach. Culture is the only method by which it is possible to make a full susceptibility test.

Histology is an invasive method which requires a least one antral biopsy and preferably two antral and two corpus biopsies. The biopsy is stained with hematoxylin and eosin, Giemsa, or silver staining. *H. pylori* is identified by the color, shape, and close relation to the mucosa and can be confirmed by immunohistochemistry using *H. pylori*-specific antibodies. The histology has shown to have a sensitivity at the same level as culture but is influenced by the size of the biopsy [14]. The number of biopsies and the location in the stomach also modify the sensitivity. The specificity of histology is lower than the specificity of the culture as histology cannot distinguish *H. pylori* from non-*pylori Helicobacter* species. The detection rates in cultures and histology varies with varying expertise of examiners. If the patient is taking proton pump inhibitor (PPI), bismuth, or antibiotics prior to gastroscopy, it might change the shape of *H. pylori* from curved rod to a coccoid form. This form is undetectable in the routine microscopy technique and requires fluorescent *in situ* hybridization, immunohistochemistry with specific antibodies to *H. pylori*, or confirmation by the 16s rRNA and 23rRNA sequencing, which are irrespective of the shape of the bacteria [16].

H. pylori urease breaks down urea to ammonia and carbon dioxide. This feature is used in the diagnostic methods “rapid urease test” (RUT) and “urea breath test” (UBT). RUT is an invasive method that preferably needs two biopsies. If the biopsy contains *H. pylori*, the release of ammonia increases the pH of the test medium, which is seen by a color change due to a pH indicator. The result of the test is fast and takes approximately ½ hour. UBT is a noninvasive method where the patient ingests ¹³C-labeled urea. If the patient is infected with *H. pylori*, orally ingested ¹³C-urea is broken down to ¹³C-labeled carbon dioxide, which is then exhaled. The sensitivity of the two tests is 75–85% for RUT and >95% for UBT. Likewise, the UBT has a higher specificity (<95%) when compared to RUT (85–95%). For both RUT and UBT, PPI and antibiotics can give false negative results. Furthermore, coccoid forms of *H. pylori* would not produce urease and would therefore give a false negative result [17].

Stool antigen test is another noninvasive method. It was first successfully described in 1997 using polyclonal antibodies [18]. Today monoclonal antibodies are used, and the sensitivity and the specificity are at the same levels as for UBT, but are preferred in special patients like children and patients with bleeding ulcers. This test can be done within ½ hour and is good for screening a patient for an infection with *H. pylori*. Despite this, antigen excretion may vary over time, and antigens may degrade while passing through the intestines, which may lead to false negative results.

The humoral antibody response to *H. pylori* can be measured by either serum IgG antibodies to *H. pylori*, which shows an ongoing or a previous infection, or by serum IgM antibodies, which shows an ongoing acute infection. *H. pylori* IgG antibodies can be detected in sputum or urine but have a much lower sensitivity and specificity than serum antibodies. Antibodies to *H. pylori* in serum can be tested by ELISA or “near patient test (NPT).” NPT uses immunochromatography or passive agglutination. A 2013 study compared the NPT and the ELISA test. The study showed that the NPT never reach 90% in sensitivity, and the frequency of false negatives and false positives were high [19]. Several tested ELISA kits showed a high specificity and sensitivity above 90%. However, the serological kits may differ considerably depending on the antigens that are included in the kit as antibodies to low-molecular-weight antigens (outer membrane antigens) decline significantly within 3 months, whereas antibodies to high-molecular-weight antigens (CagA, VacA, etc.) may stay potent for years [20]. CagA antibodies remain stable for a long period of time and can probably be useful for the detection

of *H. pylori* infections in patients with gastric cancer when other tests are negative [21]. Due to local strain distribution of *H. pylori*, the serology kits should be made by using local *H. pylori* strains, and the kits should be locally validated [21].

Gastrin and pepsinogen are compounds produced in the stomach that depend on the changes in the gastric mucosa, and the serum levels of pepsinogens are a marker of atrophic gastritis [22]. This can be combined with the *H. pylori* antibody test to predict the risk of developing gastric cancer.

Molecular methods have been of increasing interest in the field of microbiology and for detection of *H. pylori*. Polymerase chain reaction (PCR) seems to be more sensitive than any other method to detect *H. pylori* [23]. The main problem is that the method does not distinguish between live bacteria and DNA from dead bacteria. Real-time PCR (RT-PCR), which is a fast and quantitative PCR, seems to be more sensitive than classical PCR [24]. By sequencing the 16S RNA or 23S RNA region, it is possible to detect *Helicobacter* species and susceptibility to clarithromycin and tetracycline [25–27]. However, it is a more expensive and time-consuming method. A commercial kit has combined detection of *H. pylori* and susceptibility to clarithromycin in a classical PCR. However, culture is still needed for a full susceptibility testing. There are so many point mutations causing resistance to antibiotics in *H. pylori* that a full susceptibility analysis can only be detected by whole genome sequencing [28].

4. *H. pylori* susceptibility to antibiotics

During the last decade, an increased number of *H. pylori* have become resistant to antibiotics, especially to clarithromycin and levofloxacin [29]. The resistance rates to metronidazole have always been more than 15% worldwide, but the increasing resistance rates to clarithromycin and levofloxacin in some areas have become higher than 10–15%. Thus, these antibiotics are not recommended for first-line therapy of *H. pylori* without prior susceptibility testing [21]. It is common to treat *H. pylori* infections without prior susceptibility testing, and different studies show a much lower resistance rate to clarithromycin in *H. pylori* from untreated patients than in *H. pylori* from previously treated patients [30–32]. It is therefore of the greatest importance to make susceptibility testing after the first treatment failure.

The susceptibility testing of *H. pylori* can be done by various methods. The most common are dilution methods, disk diffusion, and E-test.

The dilution method is regarded to be the golden standard for susceptibility testing. A two-fold dilution row of the test antibiotic is made. A standard number of bacteria (McFarland 3) are added to each tube with antibiotics. The bacterial growth is inhibited by high concentrations of antibiotics. The first tube with bacterial growth is called the minimal inhibitory concentration (MIC). *H. pylori* should be grown for 48–72 hours under microaerobic conditions. It may be difficult to find a suitable media in which *H. pylori* grows fast enough, and the slightest contamination will grow faster than *H. pylori* and thereby spoil the susceptibility testing.

The disk diffusion test requires a small tablet of an antibiotic. The tablet is placed on the agar plate and is incubated for 3 days. After 3 days, there will be a zone around the tablet with no

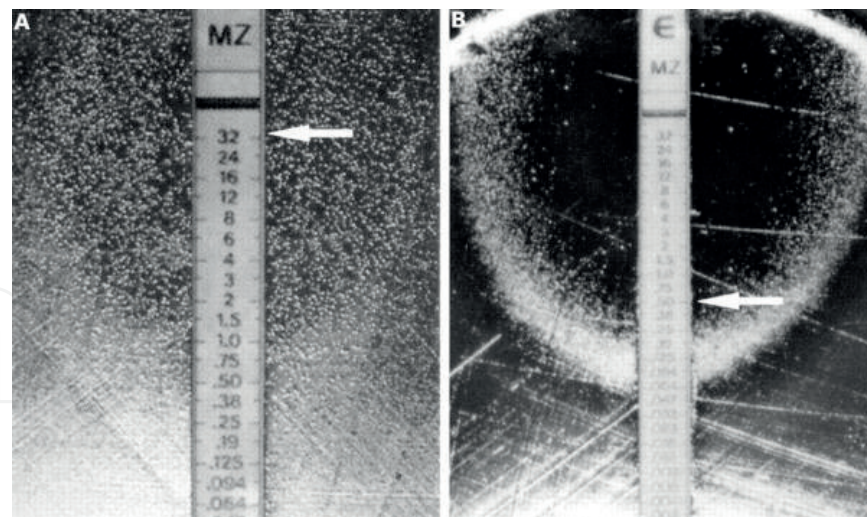


Figure 3. Reading guide for E tests. (A) Colonies of a metronidazole-resistant subpopulation in the ellipse minimum inhibitory concentration (MIC) >32; (B) trailing of microcolonies at the end point MIC 0.5 µg/ml. Warburton-Timms and McNulty [85].

growth of *H. pylori*. This is the inhibition zone, and the diameter of the zone can be translated to an MIC value, which shows whether or not the bacteria are resistant to the antibiotic. To make the susceptibility testing of *H. pylori*, a McFarland 3.0 dilution of *H. pylori* and Mueller-Hinton agar plates with 10% blood or chocolate agar plates should be used and incubated in microaerobic conditions at 37°C.

The E-test is a stripe with a concentration gradient of an antibiotic. The stripe is placed on the agar plate and is incubated for 3 days. After 3 days, there will be a droplet shape around the stripe with no growth of *H. pylori* (**Figure 3**). That concentration where *H. pylori* grows close to stripe is the MIC value [33].

5. Treatment of *H. pylori* infection

H. pylori infections are usually treated with a combination of antibiotics and nonantibiotics (proton pump inhibitor [PPI] or bismuth salts). Usually, a combination of two or three antibiotics is used, as the effect of monotherapy has been found insufficient. The most commonly used antibiotics are amoxicillin, clarithromycin, metronidazole, fluoroquinolones, tetracycline, and rifampicin (**Table 3**).

H. pylori is found in very different environments such as the gastric lumen with a relatively low pH, in between the epithelial cells and on the basement membrane with a neutral pH but protected as intracellular microorganisms. When choosing antibiotics, it is important to select antibiotic to which *H. pylori* is sensitive and is active in all the environmental niches where *H. pylori* occurs. It is also important to look at the duration of the efficacy of antibiotics to keep stable levels above the minimal inhibitory concentrations.

PPI in standard doses do not have antibacterial effect on *H. pylori*, but 5–10 times higher doses have a direct effect on *H. pylori*. Bismuth salts binds to the surface of *H. pylori* but have

Group	Preparation
Antibiotics	Amoxicillin
	Clarithromycin
	Metronidazole
	Tetracycline
	Levofloxacin
	Ciprofloxacin
	Rifampicin
Nonantibiotics	PPI
	Bismuth nitrate
	Bismuth citrate
	Bismuth subsalicylate
	H ₂ blocker

Table 3. Commonly used antibiotics and nonantibiotics for treatment of *H. pylori* infections.

a relatively little antibacterial effect. However, bismuth salts affect the respiratory chain at the same points as metronidazole and thereby reverts metronidazole resistance in *H. pylori* and thus becomes sensitive to metronidazole.

6. Prevalence of *H. pylori* resistance to antibiotics

When analyzing different studies around the world, the primary resistance rate for *H. pylori* varies. The highest rate of primary metronidazole (MTZ) resistance is found in Africa (52%) followed by South America (49%) and Asia (43%). The lowest resistance rate is found in Europe (35%). The highest primary resistance rates for clarithromycin and levofloxacin are found in South America (20 and 27%) while the lowest rates are found in Europe (12 and 10%) [30–32, 34–67]. There is a significantly ($p < 0.001$) higher risk of primary metronidazole and levofloxacin resistance in Asian when compared to Europe.

The high rate of metronidazole resistance seen in developing countries may be due to the high use of metronidazole for treatment of parasites and gynecological infections [62, 68]. It is therefore likely that the patients who are treated for *H. pylori* with metronidazole for the first time are resistant for this treatment. It is recommended to use bismuth therapy together with metronidazole in the first-line treatment in areas with high metronidazole resistance [21].

The high resistance rates for clarithromycin and levofloxacin in South America, Africa, and Asia can be due to the use of huge amounts of antibiotics in general [69]. Typically, the diagnostics are not precise, and the patients are treated with more a broad spectrum of antibiotics for a longer period. This can lead to a faster development of resistance in *H. pylori* [70].

A large multinational study tested *H. pylori* resistance in 18 European countries [29]. All 18 countries used E-test for the susceptibility testing and only tested patients who had never been treated for *H. pylori* before. In total, 2204 people were included in the study, and the resistance rate for adults were 18% for clarithromycin, 14% for levofloxacin, and 35% for metronidazole. They found a significant association between the use of only long-acting macrolides and clarithromycin resistance. The levofloxacin resistance was significantly associated with the use of quinolone.

The prevalence of *H. pylori* resistance to antibiotics was tested in Denmark in 1997, 1998–2004, and 2013 [71–73]. Throughout the years, the resistance for clarithromycin has increased from 0% in 1997 to 53% in 2013, and likewise, the resistance for metronidazole increased from 20 to 74% [12–14]. None of the studies mention whether or not the patients have had *H. pylori* eradication therapy prior to testing or not, which might explain the huge increase in resistance.

6.1. Effect of antibiotic treatment on *H. pylori* resistance rates

International guidelines recommend first line of treatment of *H. pylori* infections with 10 days of triple therapy (PPI, clarithromycin, and metronidazole or amoxicillin). If this fails, a treatment with four types of medicine (PPI, bismuth subsalicylate, tetracycline, and metronidazole) for 2 weeks is recommended. After treatment failure for the second time, it is recommended to perform a gastroscopy and susceptibility testing for *H. pylori* [21].

The primary and secondary resistance rate for *H. pylori* has only been described in eight studies [30, 32, 40, 43, 58, 65, 66, 74]. By using “Review Manager 5.3,” it is possible to compare the studies via Forest plots. The meta-analyses show that the secondary resistance is significantly higher ($p < 0.001$) than the primary.

The meta-analysis shows a high increasing resistance rate for all three antibiotics when the patient had been treated for *H. pylori* previously. The high and increasing resistance rates to metronidazole, clarithromycin, and levofloxacin make it uncertain that these antibiotics should be recommended as the first-line therapy of *H. pylori* infections without prior endoscopy and susceptibility testing (Figure 4A–C).

6.2. Vaccine

Another way to overcome *H. pylori* infections is with a vaccine. In the past couple of years, many studies have investigated developing an effective and safe vaccine. The development of an effective vaccine is complicated by the noninvasive nature of *H. pylori*. It stays in the lumen of the stomach and does not cross the epithelium. Therefore, the vaccine should affect T helper memory cells, which are required to stay in the lumen during a *H. pylori* infection [75].

Appropriate bacterial antigens, safe and effective adjuvants, and a route of delivery are required for developing a vaccine. For the bacterial antigen, most studies use urease, but other antigens are investigated for example Cag L. The CagL is a protein essential for the pathogenesis of *H. pylori*. It binds to integrins in the mucosa and triggers the release of the carcinogen CagA to the host cells

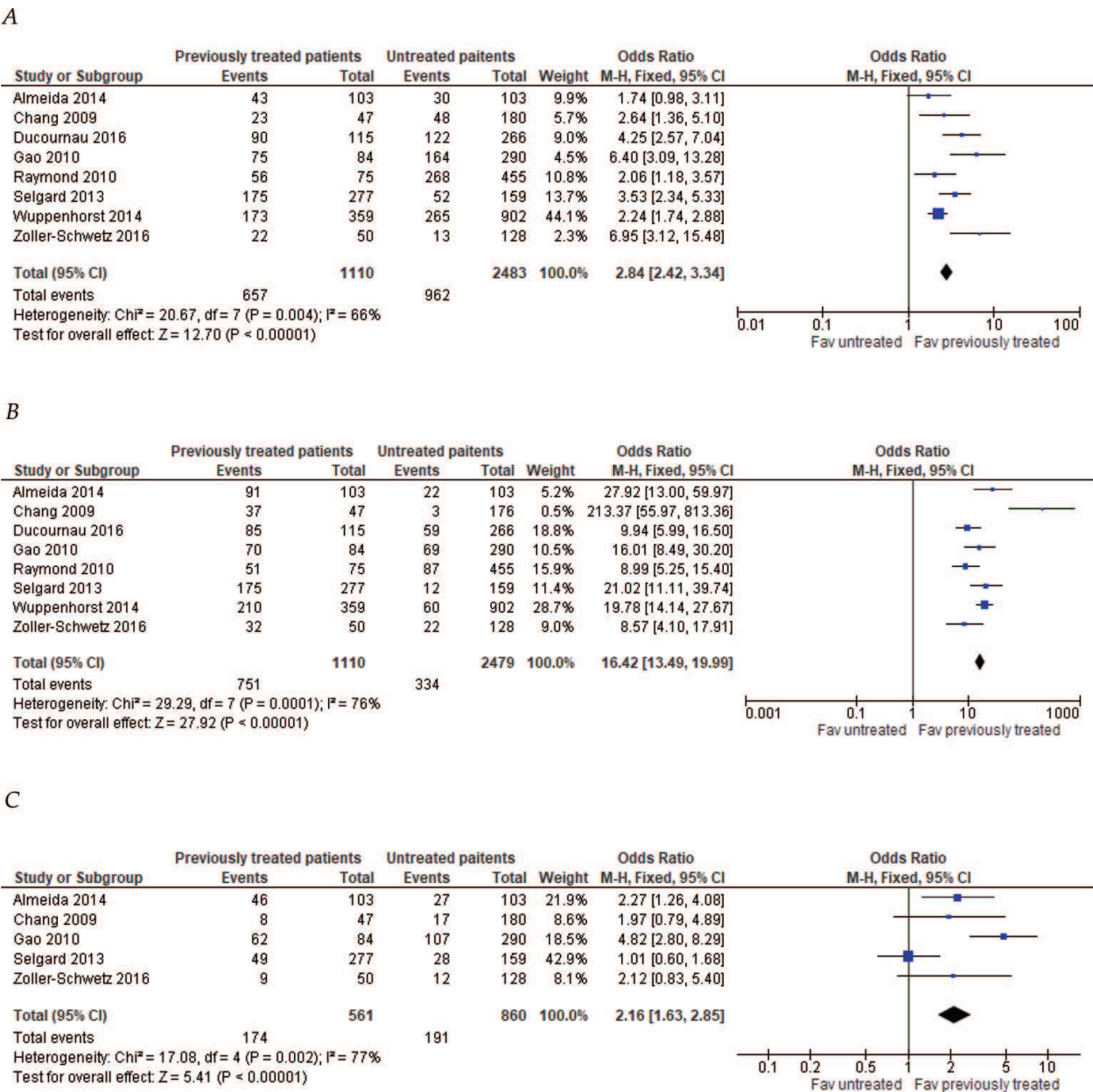


Figure 4. Meta-analysis for MTZ (A), CLR (B), and LEV (C). For all three antibiotics, there is a higher odds ratio for resistance if the patient is previously treated for infection with *H. pylori*.

through the type IV secretin system. CagL also introduces an IL-8 response, which causes inflammation [76]. The use of CagL in a subunit vaccine was investigated by Choudhari et al. in 2013 [75]. The study showed that CagL was stable in pH 4–6 and that sucrose enhances the stability.

The use of heat shock proteins in a vaccine introduced protective immunity without requiring the addition of an adjuvant. The protection, however, is not optimal because sterilizing immunity is not obtained, which is shown in a study from 2014 [77].

A derivate of the cholera toxin (CTA1-DD) and safe nontoxic mutants of *Escherichia coli* heat labile toxin (dm2T) have also been tested as potential adjuvants. CTA1-DD enhances the Th1 and Th17 immunity and reduces the bacterial colonization by three- to eight-fold [78]. The use of dm2T was equally as effective as the gold standard *H. pylori* vaccine containing cholera toxin [79].

The routes of delivery that have been tested are sublingual, intranasal, respiratory, and oral [79]. A study on humans from China (2015) tested a vaccine based on a urease B subunit and heat-labile enterotoxin B subunit (gene derived from *E. coli* H44815) [80]. The vaccine was taken orally three times (day 0, 14, and 28). This study showed a vaccine efficacy of 71.8% in the first year, 55% in the second year, and 55.8% in the third year after vaccinations. Even though these findings are excellent, a 100% effective vaccine is still not developed. More studies and longer time follow-ups are needed before a fully effective vaccine is on the market. If a fully effective vaccine is made, it would be the best health measure against *H. pylori* infections and gastric cancer.

7. Discussion

The human gastric microbiota may be difficult to estimate since samples for microbiome investigations often are contaminated with oral bacterial flora during gastroscopy. And the studies in these fields do not make any attempt to remove the oral contamination prior to sequencing. Histological examination of biopsies reveals *H. pylori* as the only bacteria in close relation to the epithelial cells in the gastric mucosa. When *H. pylori* is seen in stomach samples, there is always a strong humoral and cellular immune response to *H. pylori* and it thereby fulfills the criteria for a true infection but also a colonization. This has not been shown for any other bacteria.

Thus, in noncancer patients, *H. pylori* seems to be the gastric microbiota. In patients with gastric cancer, there may be a different situation as the mucosa is disintegrated and an overgrowth of intestinal bacteria is common. However, it remains to be shown that the intestinal bacteria adhere to the gastric mucosa and cause a local immune response. It is, therefore, believed that *H. pylori* is still the most important gastric pathogen.

An increasing resistance to antibiotics in *H. pylori* has been seen worldwide especially to metronidazole, clarithromycin, and levofloxacin. This is a worrying development as it may interfere with our recommendations for primary treatment of *H. pylori* without susceptibility testing. It is a question how fast the resistance occurs. Should susceptibility testing be done after first treatment failure or can it wait until the second treatment failure as recommended? At least the resistance rates are much higher in previously treated patients than in untreated patients.

Due to the high resistant rates, it is necessary to perform a susceptibility test before starting the treatment. The advantages would be a better and maybe quicker eradication of the *H. pylori* infection. Disadvantages of early susceptibility testing are the cost and time of the analyses. Biopsies are an invasive method and may often be painful for the patient. Furthermore, it takes up to 14 days before a full susceptibility test is completed, so the real treatment starts approximately 2 weeks after the doctor confirms the presence of *H. pylori*. By this time, the patient could have been done with the first line of treatment. In the short perspective, a quick susceptibility test would be very time consuming, but in the long perspective, it might save the patient from several treatments and prevent the relapse of the *H. pylori* infection. But it also gives a better overview on how quickly *H. pylori* develops resistance to the recommended treatment.

When detecting *H. pylori*, the best would be a quick a method that was as quick as PCR but also made it possible to have a full susceptibility test incorporated. New primers for detecting

antibiotic resistance are in progress, but the problem is that there are many different mutations leading to the same resistance profile. *H. pylori* only develops antibiotic resistance by mutation in the genome. For MTZ, mutations in at least nine different genes are known to contribute to MTZ resistance [13]. If the detecting of MTZ resistance should be made by PCR, it would be necessary to perform the PCR with many different primers all looking for one specific mutation. In theory, this would be the most sensitive way to find MTZ resistance, but in practice, it would be almost impossible, take a lot of time, and would be expensive.

Due to the enormous amount of mutations leading to antibiotic resistant, the culture and susceptibility testing done by E-test is still the best and most economical way.

The increasing resistant rates to the most commonly used antibiotics raises the question of whether other antibiotics or combinations of antibiotic and nonantibiotic should be used for primary treatment of *H. pylori* infections without susceptibility testing. Bismuth compounds in standard doses, proton pump inhibitors, and acid suppressing compounds in high doses may convert the MTZ resistance [81]. This makes MTZ useful in combination with these compounds, especially the bismuth compounds, which have been shown in clinical studies [21]. Nonantibiotics such as neuroleptics and other compounds acting on the central nerves system have anti-*H. pylori* effect *in vitro* [82] and compounds without effect on the central nervous system may be candidates for alternative treatment. Herbs like broccoli and green tee have some effect on *H. pylori* and may in combination with antibiotics and nonantibiotics be candidates for treatment in the future [83].

8. Conclusion

H. pylori is the most important gastric pathogen and may constitute the true gastric microbiota. It is, therefore, important to follow the development of resistance in *H. pylori* to antibiotics. With the increased resistance of *H. pylori* to metronidazole, clarithromycin, and levofloxacin, it may be doubtful if these antibiotics can be recommended as primary treatment without susceptibility testing.

Conflict of interest

The authors declare no conflicts of interests.

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References

- [1] Ursell LK, Metcalf JL, Parfrey LW, et al. Defining the human microbiome. *Nutrition Reviews*. 2012;**70**(Suppl 1):S38-S44
- [2] Jackson MA, Bonder MJ, Kuncheva Z, et al. Detection of stable community structures within gut microbiota co-occurrence networks from different human populations. *PeerJ*. 2018;**6**
- [3] Jorgensen JH, Pfaller MA, Karen C, et al. *Manual of Clinical Microbiology*. 11th ed. Washington DC: ASM Press; 2015
- [4] Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, et al. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. *Scientific Reports*. 2015;**4**(1):4202
- [5] Wang L, Zhou J, Xin Y, Geng C, et al. Bacterial overgrowth and diversification of microbiota in gastric cancer. *European Journal of Gastroenterology & Hepatology*. 2016;**28**(3):261-266
- [6] Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proceedings of the National Academy of Sciences*. 2006;**103**(3):732-737
- [7] Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *The ISME Journal*. 2011;**5**(4):574-579
- [8] Yang I, Nell S, Suerbaum S. Survival in hostile territory: the microbiota of the stomach. *FEMS Microbiology Reviews*. 2013;**37**(5):736-761
- [9] Liu X, Nie W, Liang J, et al. Interaction of *Helicobacter Pylori* with other microbiota species in the development of gastric cancer. *Archives of Clinical Microbiology*. 2017;**8**(2)
- [10] Carroll AC, Wong A. Plasmid persistence: Costs, benefits and the plasmid paradox. *Canadian Journal of Microbiology*. May 2018;**64**(5):293-304. cjm-2017-0609
- [11] Dorward DW, Garon CF. DNA-binding proteins in cells and membrane blebs of *Neisseria gonorrhoeae*. *Journal of Bacteriology*. 1989;**171**(8):4196-4201
- [12] Durão P, Balbontín R, Gordo I. Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends in Microbiology*. Aug 2018;**26**(8):677-691
- [13] Arslan N, Yılmaz Ö, Demiray-Gürbüz E. Importance of antimicrobial susceptibility testing for the management of eradication in *Helicobacter pylori* infection. *World Journal of Gastroenterology*. 2017;**23**(16):2854
- [14] Bytzer P, Dahlerup JF, Eriksen JR, et al. Diagnosis and treatment of *Helicobacter pylori* infection. *Danish Medical Bulletin*. 2011;**58**(4):1-5
- [15] Cuchi E, Forné M, Quintana S. Comparison of two transport media and three culture media for primary isolation of *Helicobacter pylori* from gastric biopsies. *European Society of Clinical Microbiology and Infectious Diseases*. 2002;**8**:609-610
- [16] Patel SK, Pratap CB, Jain AK, et al. Diagnosis of *Helicobacter pylori*: What should be the gold standard? *World Journal of Gastroenterology*. 2014;**20**(36):12847-12859

- [17] Koletzko S. Noninvasive diagnostic tests for *Helicobacter pylori* infection in children. *Canadian Journal of Gastroenterology*. 2005;**19**(7):433-439
- [18] Makristathis A, Pasching E, Schütze K, et al. Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. *Journal of Clinical Microbiology*. 1998;**36**(9):2772-2774
- [19] Burucoa C, Delchier JC, Courillon-Mallet A, et al. Comparative evaluation of 29 commercial *Helicobacter pylori* serological kits. *Helicobacter*. 2013;**18**(3):169-179
- [20] Andersen LP, Espersen F, Souckova A, et al. Isolation and preliminary evaluation of a low-molecular-mass antigen preparation for improved detection of *Helicobacter pylori* immunoglobulin G antibodies. *Clinical and Diagnostic Laboratory Immunology*. 1995;**2**(2):156-159
- [21] Malfertheiner P, Megraud F, O'morain CA, et al. Management of *Helicobacter pylori* infection—the Maastricht V/florence consensus report. *Gut*. 2017;**66**:6-30
- [22] Shimoyama T, Oyama T, Matsuzaka M, et al. Comparison of a stool antigen test and serology for the diagnosis of *Helicobacter pylori* infection in mass survey. *Helicobacter*. 2009;**14**(2):87-90
- [23] Cosgun Y, Yildirim A, Yucel M, et al. Evaluation of invasive and noninvasive methods for the diagnosis of *Helicobacter Pylori* infection. *Asian Pacific Journal of Cancer Prevention*. 2016;**17**(12):5265-5272
- [24] Monno R, Giorgio F, Carmine P, et al. *Helicobacter pylori* clarithromycin resistance detected by Etest and TaqMan real-time polymerase chain reaction: A comparative study. *APMIS*. 2012;**120**(9):712-717
- [25] Redondo JJ, Keller PM, Zbinden R, et al. A novel RT-PCR for the detection of *Helicobacter pylori* and identification of clarithromycin resistance mediated by mutations in the 23S rRNA gene. *Diagnostic Microbiology and Infectious Disease*. 2018;**90**(1):1-6
- [26] Dadashzadeh K, Milani M, Rahmati M, et al. Real-time PCR detection of 16S rRNA novel mutations associated with *Helicobacter pylori* tetracycline resistance in Iran. *Asian Pacific Journal of Cancer Prevention*. 2014;**15**(20):8883-8886
- [27] Pastukh N, Binyamin D, On A, et al. GenoType® HelicoDR test in comparison with histology and culture for *Helicobacter pylori* detection and identification of resistance mutations to clarithromycin and fluoroquinolones. *Helicobacter*. 2017;**22**(6):e12447
- [28] Draper JL, Hansen LM, Bernick DL, et al. Fallacy of the unique genome: Sequence diversity within single *Helicobacter pylori* strains. Fraser CM, editor. *MBio*. 2017;**8**(1):e02321-e02316
- [29] Megraud F, Coenen S, Versporten A, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut*. 2013;**62**(1):34-42
- [30] Gao W, Cheng H, Hu F, et al. The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China. *Helicobacter*. 2010;**15**:460-466
- [31] Selgrad M, Meile J, Bornschein J, et al. Antibiotic susceptibility of *Helicobacter pylori* in central Germany and its relationship with the number of eradication therapies. *European Journal of Gastroenterology & Hepatology*. 2013;**25**(11):1257-1260

- [32] Almeida N, Romãozinho JM, Donato MM, et al. *Helicobacter pylori* antimicrobial resistance rates in the central region of Portugal. *Clinical Microbiology and Infection*. 2014;**20**(11): 1127-1133
- [33] Ogata SK, Gales AC, Kawakami E. Antimicrobial susceptibility testing for *Helicobacter pylori* isolates from Brazilian children and adolescents: Comparing agar dilution, e-test, and disk diffusion. *Brazilian Journal of Microbiology*. 2014;**45**(4):1439-1448
- [34] Ahmad N, Zakaria WR, Mohamed R. Analysis of antibiotic susceptibility patterns of *Helicobacter pylori* isolates from Malaysia. *Helicobacter*. 2011;**16**:47-51
- [35] Ang TL, Fock KM, Ang D, et al. The changing profile of *Helicobacter pylori* antibiotic resistance in Singapore: A 15-year study. *Helicobacter*. 2016;**21**(4):261-265
- [36] Binh TT, Shiota S, Nguyen LT, et al. The incidence of primary antibiotic resistance of *Helicobacter pylori* in Vietnam. Nixon AE, editor. *Journal of Clinical Gastroenterology*. 2013; **47**(3):233-238
- [37] Boehnke KF, Valdivieso M, Bussalleu A, et al. Antibiotic resistance among *Helicobacter pylori* clinical isolates in Lima, Peru. *Infection and Drug Resistance*. 2017;**10**:85-90
- [38] Bouihat N, Burucoa C, Benkirane A, et al. *Helicobacter pylori* primary antibiotic resistance in 2015 in Morocco: A phenotypic and genotypic prospective and multicenter study. *Microbial Drug Resistance*. 2016;**23**(6):727-732
- [39] Caliskan R, Tokman HB, Erzin Y, et al. Antimicrobial resistance of *Helicobacter pylori* strains to five antibiotics, including levofloxacin, in Northwestern Turkey. *Revista da Sociedade Brasileira de Medicina Tropical*. 2015;**48**(3):278-284
- [40] Chang WL, Sheu BS, Cheng HC, et al. Resistance to metronidazole, clarithromycin and levofloxacin of *Helicobacter pylori* before and after clarithromycin-based therapy in Taiwan. *Journal of Gastroenterology and Hepatology*. 2009;**24**(7):1230-1235
- [41] Cheng A, Sheng WH, Liou JM, et al. Comparative in vitro antimicrobial susceptibility and synergistic activity of antimicrobial combinations against *Helicobacter pylori* isolates in Taiwan. *Journal of Microbiology, Immunology, and Infection*. 2015;**48**(1):72-79
- [42] Cuadrado-Lavín A, Salcines-Caviedes JR, Carrascosa MF, et al. Antimicrobial susceptibility of *Helicobacter pylori* to six antibiotics currently used in Spain. *The Journal of Antimicrobial Chemotherapy*. 2012;**67**(1):170-173
- [43] Ducournau A, Bénéjat L, Sifré E, et al. *Helicobacter pylori* resistance to antibiotics in 2014 in France detected by phenotypic and genotypic methods. *Clinical Microbiology and Infection*. 2016;**22**(8):715-718
- [44] Eisig JN, Silva F, Barbuti RC, et al. *Helicobacter pylori* antibiotic resistance in Brazil: Clarithromycin is still a good option. *Arquivos de Gastroenterologia*. 2011;**48**(4):261-264
- [45] Farshad S, Alborzi A, Japoni A, et al. Antimicrobial susceptibility of *Helicobacter pylori* strains isolated from patients in Shiraz, Southern Iran. *World Journal of Gastroenterology*. 2010;**16**(45):5746-5751

- [46] Dargiene G, Kupcinskas J, Jonaitis L, et al. Primary antibiotic resistance of *Helicobacter pylori* strains among adults and children in a tertiary referral centre in Lithuania. *APMIS*. 2017
- [47] Goh KL, Navaratnam P. High *Helicobacter pylori* resistance to metronidazole but zero or low resistance to clarithromycin, levofloxacin, and other antibiotics in Malaysia. *Helicobacter*. 2011;**16**(3):241-245
- [48] Gościński G, Biernat M, Grabińska J, et al. The antimicrobial susceptibility of *Helicobacter pylori* strains isolated from children and adults with primary infection in the Lower Silesia Region, Poland. *Polish Journal of Microbiology*. 2014;**63**(1):57-61
- [49] Gunnarsdottir AI, Gudjonsson H, Hardardottir H, et al. Antibiotic susceptibility of *Helicobacter pylori* in Iceland. *Infectious Diseases. (Auckland)*. 2017;**49**(9):647-654
- [50] Karczewska E, Wojtas-Bonior I, Sito E, et al. A primary and secondary clarithromycin, metronidazole, amoxicillin and levofloxacin resistance to *Helicobacter pylori* in southern Poland. *Pharmacological Reports*. 2011;**63**(3):799-807
- [51] Kostamo P, Veijola L, Oksanen A, et al. Recent trends in primary antimicrobial resistance of *Helicobacter pylori* in Finland. *International Journal of Antimicrobial Agents*. 2011;**37**(1): 22-25
- [52] Kupcinskas L, Rasmussen L, Jonaitis L, et al. Evolution of *Helicobacter pylori* susceptibility to antibiotics during a 10-year period in Lithuania. *APMIS*. 2013;**121**(5):431-436
- [53] Larsen AL, Ragnhildstveit E, Moayeri B, et al. Resistance rates of metronidazole and other antibacterials in *Helicobacter pylori* from previously untreated patients in Norway. *APMIS*. 2013;**121**(4):353-358
- [54] Ben Mansour K, Buruoa C, Zribi M, et al. Primary resistance to clarithromycin, metronidazole and amoxicillin of *Helicobacter pylori* isolated from Tunisian patients with peptic ulcers and gastritis: a prospective multicentre study. *Annals of Clinical Microbiology and Antimicrobials*. 2010;**9**(1):22
- [55] Miftahussurur M, Syam AF, Nusi IA, et al. Surveillance of *Helicobacter pylori* antibiotic susceptibility in Indonesia: Different resistance types among regions and with novel genetic mutations. *PLoS One*. 2016;**11**(12):1-17
- [56] O'Connor A, Taneike I, Nami A, et al. *Helicobacter pylori* resistance rates for levofloxacin, tetracycline and rifabutin among Irish isolates at a reference centre. *Irish Journal of Medical Science*. 2013:1-3
- [57] Quek C, Pham ST, Tran KT, et al. Antimicrobial susceptibility and clarithromycin resistance patterns of *Helicobacter pylori* clinical isolates in Vietnam. *F1000Research*. 2016;**5**(0):671
- [58] Raymond J, Lamarque D, Kalach N, et al. High level of antimicrobial resistance in French *Helicobacter pylori* isolates. *Helicobacter*. 2010;**15**(1):21-27
- [59] Saracino IM, Zullo A, Holton J, et al. High prevalence of primary antibiotic resistance in *Helicobacter pylori* isolates in Italy. *Journal of Gastrointestinal and Liver Diseases*. 2012;**21**(4):363-365

- [60] Seck A, Burucoa C, Dia D, et al. Primary antibiotic resistance and associated mechanisms in *Helicobacter pylori* isolates from Senegalese patients. *Annals of Clinical Microbiology and Antimicrobials*. 2013;**12**:3
- [61] Shiota S, Reddy R, Alsarraj A, et al. Antibiotic resistance of *Helicobacter pylori* among male United States veterans. *Clinical Gastroenterology and Hepatology*. 2015;**13**(9):1616-1624
- [62] Teh X, Khosravi Y, Lee WC, et al. Functional and molecular surveillance of *Helicobacter pylori* antibiotic resistance in Kuala Lumpur. *PLoS One*. 2014;**9**(7)
- [63] Torres-Debat ME, Pérez-Pérez G, Olivares A, et al. Antimicrobial susceptibility of *Helicobacter pylori* and mechanisms of clarithromycin resistance in strains isolated from patients in Uruguay. *Revista Española de Enfermedades Digestivas*. 2009;**101**(11):757-762
- [64] Korn VR, Gumnarai P, Ratanachu-ek T, et al. Nationwide survey of *Helicobacter pylori* antibiotic resistance in Thailand. *Diagnostic Microbiology and Infectious Disease*. 2013;**77**(4):346-349
- [65] Wuppenhorst N, Draeger S, Stuger HP, et al. Prospective multicentre study on antimicrobial resistance of *Helicobacter pylori* in Germany. *The Journal of Antimicrobial Chemotherapy*. 2014;**69**(11):3127-3133
- [66] Zollner-Schwetz I, Leitner E, Plieschnegger W, et al. Primary resistance of *Helicobacter pylori* is still low in Southern Austria. *International Journal of Medical Microbiology*. 2016;**306**(4):206-211
- [67] Wu IT, Chuah SK, Lee CH, et al. Five-year sequential changes in secondary antibiotic resistance of *Helicobacter pylori* in Taiwan. *World Journal of Gastroenterology*. 2015;**21**(37):10669-10674
- [68] Oleastro M, Cabral J, Ramalho PM, et al. Primary antibiotic resistance of *Helicobacter pylori* strains isolated from Portuguese children: A prospective multicentre study over a 10 year period. *The Journal of Antimicrobial Chemotherapy*. 2011;**66**(10):2308-2311
- [69] Van Boeckel TP, Gandra S, Ashok A, et al. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases*. 2014;**14**(8):742-750
- [70] WHO. Antimicrobial Resistance. Global Report on Surveillance. Geneva: World Health Organization; 2014. pp. 383-394
- [71] Hartzen SH, Andersen LP, Bremmelgaard A, et al. Antimicrobial susceptibility testing of 230 *Helicobacter pylori* strains: Importance of medium, inoculum, and incubation time. *Antimicrobial Agents and Chemotherapy*. 1997;**41**(12):2634-2349
- [72] Rasmussen L. *Helicobacter pylori* [PhD thesis]. Copenhagen; 2013
- [73] Petersen AM, Gjøde P, Vinge OD, et al. *Helicobacter pylori* antimicrobial resistance and risk factors in Denmark 1998-2004: No need for concern? *Helicobacter*. 2006;**11**(3):210-211
- [74] Selgrad M, Tammer I, Langner C, et al. Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of *Helicobacter pylori* infection. *World Journal of Gastroenterology*. 2014;**20**(43):16245-16251

- [75] Choudhari SP, Pendleton KP, Ramsey JD, et al. A systematic approach toward stabilization of CagL, a protein antigen from *Helicobacter Pylori* that is a candidate subunit vaccine. *Journal of Pharmaceutical Sciences*. 2013;**102**:2508-2519
- [76] Kwok T, Zabler D, Urman S, et al. *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature*. 2007;**449**(7164):862-866
- [77] Chionh YT, Arulmuruganar A, Venditti E, et al. Heat shock protein complex vaccination induces protection against *Helicobacter pylori* without exogenous adjuvant. *Vaccine*. 2014;**32**:2350-2358
- [78] Nedrud JG, Bagheri N, Schön K, et al. Subcomponent vaccine based on CTA1-DD adjuvant with incorporated UreB class II peptides stimulates protective *Helicobacter pylori* immunity. Ho PL, editor. *PLoS One*. 2013;**8**(12):e83321
- [79] D'Elia MM, Czinn SJ. Immunity, Inflammation, and Vaccines for *Helicobacter pylori*. *Helicobacter*. 2014;**19**(S1):19-261
- [80] Zeng M, Mao XH, Li JX, et al. Efficacy, safety, and immunogenicity of an oral recombinant *Helicobacter pylori* vaccine in children in China: A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2015;**386**(10002):1457-1464
- [81] Chen M, Jensen B, Zhai L, et al. Nizatidine and omeprazole enhance the effect of metronidazole on *Helicobacter pylori* in vitro. *International Journal of Antimicrobial Agents*. 2002;**19**(3):195-200
- [82] Kristiansen JE, Justesen T, Hvidberg EF, et al. Trimipramine and other antipsychotics inhibit *Campylobacter pylori* in vitro. *Pharmacology & Toxicology*. 1989;**64**(4):386-388
- [83] Fahey JW, Stephenson KK, Wallace AJ. Dietary amelioration of *Helicobacter* infection. *Nutrition Research*. 2015;**35**(6):461-473
- [84] Rahbar M, Mardanpur K, Tavafzadeh R. Imprint cytology: A simple, cost effectiveness analysis for diagnosing *Helicobacter pylori*, in west of Iran. *Medical journal of the Islamic Republic of Iran*. 2012;**26**(1):12-16
- [85] Warburton-Timms V, McNulty C. Role of screening agar plates for in vitro susceptibility testing of *Helicobacter pylori* in a routine laboratory setting. *Journal of Clinical Pathology*. 2001;**54**(5):408-411